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Interactions of Chlorine-Produced Oxidants Salinity, and a Protistan Parasite in Affecting Lethal and Sublethal Physiological Effects in the Eastern or American Oyster

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16. ABSTRACT

Results from this study have generally indicated the significant interactive effects of salinity and CPO exposure in affecting oyster mortalities resulting from P. marinus infection.

Comparisons of control oysters in high-and low-salinity exposures generally indicated that low-salinity conditions resulted in exposure of oysters to water which was reduced in both salt content (major ions) and phytoplankton density (as food availability was reduced by 66%). Biodeposition rates (or feeding rates) were significantly reduced in low-salinity-exposed oysters, probably related to less food availability.

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CHAPTER 37

Interactions of Chlorine-Produced Oxidants, Salinity, and A Protistan Parasite in Affecting Lethal and Sublethal Physiological Effects in the Eastern or American Oyster

Geoffrey I. Scott, Edward O. Oswald, Tommy I. Sammons, Douglas S. Baughman, and Douglas P. Middaugh

The discharge of chlorine into estuarine habitats may seriously affect organisms residing there.[1,2] Several studies[3–6] have shown that the ability of oysters to survive chlorine and bromoform exposure is clearly dependent on the physiological condition of the organism. In comparisons of spring and summer chronic exposures at identical chlorine concentrations, salinities, and temperatures, oyster survival rates were three to four times greater during the spring (prespawning period) than in the summer (postspawning period).[4] Additionally, summer respiration rates were significantly higher than spring values in chlorine-exposed oysters.[6] Both spring and summer groups were infected with the protistan parasite, *Perkinsus marinus*.

Studies on the epizootiology of *P. marinus* have indicated that this parasite is basically a summer disease causing peak mortalities in the eastern or American oyster, *Crassostrea virginica*, of greater than 50% in August and September when water temperatures and salinity values are maximal.[7,8] Salinity has been shown to be an important factor affecting oyster mortalities; *P. marinus* infection intensities and associated mortalities are greatest in high-salinity waters.[9,10] Low-salinity exposure tends to depress infection intensities and significantly enhance oyster survival.[11–14]

As earlier discussions[4–6] indicated, oysters are much more susceptible to chlorination exposure during the summer months. This increased summer susceptibility to chlorine exposure may be caused by lessened resistance in the oyster, resulting from *P. marinus* infections. Several authors[15,16] have described the interactions between increased levels of pollution and increased incidences of parasitism and disease in marine organisms.

We investigated the interactions of chlorination exposure and salinity in affecting lethal and sublethal responses in oysters, *C. virginica*, infected with the protistan parasite, *P. marinus*.

MATERIALS AND METHODS

Collection and Acclimation

Adult oysters (6–12 cm in height) were collected from Leadenwah Creek (latitude, N 32°36′; longitude, W 80°15′), a large tidal tributary of the North Edisto River Estuary in South Carolina on June 21, 1982. Following collection, oysters were immediately transported to the laboratory where they were scrubbed and all fouling organisms were removed. After numbering and measuring the height of individual oysters, 120 animals were placed in each test aquarium (circular tanks: 152.4-cm radius × 30-cm depth with a working volume on 310 L) and were acclimated for 39 d in unfiltered seawater pumped in daily from the Stono River at high tide. Tanks were cleaned daily during acclimation.

Following acclimation, oysters in each tank were exposed for 60 d to one of several treatments described in Table I. Tests were conducted from August through October 1982.

Exposure Conditions

Low-salinity water (treatment E) was prepared by diluting incoming ambient estuarine water with groundwater from a shallow well. Normal ambient salinity at high tide ranged from 21 to 25 parts per thousand (ppt). Low-salinity water of 8 to 10 ppt was desired, which required approximately a 40:60% dilution of estuarine water:groundwater.

Preparations of chlorinated seawater were made at both low (8–10 ppt) and high (21–25 ppt) salinities by daily addition of NaOCl (Fisher Reagent Grade, 5%) to each tank at appropriate concentrations and dilutions. Nominal concentrations were 1.00, 0.56, and 0.10 mg/L in both high-salinity (treatments B, C, and D, respectively) and low-salinity (treatments F, G, and H, respectively) waters. Oysters were also exposed to ambient chlorine-free high-salinity and low-salinity estuarine water (treatments A and E, respectively) to serve as indicators of natural or control conditions.

A total of 120 oysters were placed in each test aquarium and were exposed to each treatment for 60 d. Oysters fed on phytoplankton in the seawater and were exposed to the ambient light:dark cycle (14:10 h) and seawater temperatures. Water changes were made and test aquaria were cleaned daily throughout the experiment.

Test Parameters Measured

During each experiment, the following measurements were used to assess the physiological effects of each treatment on adult oysters:

Table I. Description of Treatment Codes and Physical and Chemical Parameters Measured in Each of the Various Salinity and Chlorination Treatments used in This Study. Statistical measurements included calculations of mean ($\bar{x}$) and standard error (S.E.). Single (*) or double (**) asterisks indicate measured CPO concentrations in each low-salinity CPO treatment were significantly ($^b p < 0.05$) or very significantly ($p < 0.01$) different from similar high-salinity CPO treatments.

Treatment Code	Treatment Description	Measured CPO Concentration (mg/L)[a]		Exposure Salinity (ppt)[b]		Exposure Temperature (°C)[c]	
		$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.
A	High-salinity control	0.00	0.000	22.6	0.42	24.2	0.40
B	High salinity 1.00-mg/L NaOCl[d] concentration	0.06	0.007	22.9	0.21	24.2	0.40
C	High salinity 0.56-mg/L NaOCl concentration	0.03	0.005	22.7	0.04	24.0	0.39
D	High salinity 0.10-mg/L NaOCl concentration	0.01	0.003	22.9	0.21	24.0	0.39
E	Low-salinity control	0.00	0.000	9.2	0.18	23.9	0.38
F	Low salinity 1.00-mg/L NaOCl concentration	0.11**	0.008	9.3	0.18	23.6	0.39
G	Low salinity 0.56-mg/L NaOCl concentration	0.06**	0.007	9.6	0.59	23.6	0.39
H	Low salinity 0.10-mg/L NaOCl concentration	0.02*	0.004	9.0	0.17	23.6	0.39

[a]Sample size (n) ranged from 36 to 38.
[b]Sample size ranged from 57 to 58.
[c]Sample size ranged from 51 to 52.
[d]NaOCl refers to nominal NaOCl concentrations added.

1. Survival and mortality percentages were recorded daily, using three replicates per treatment.

2. Chlorine-produced oxidant (CPO) concentrations (free plus combined chlorine, bromine, and other residual oxidants) were measured with a Fisher Titrator (Model 397). Phenylarsine oxide and pH 4 buffer were standard Fisher reagents. The 5% potassium iodide (KI) solution and chlorine (Fisher NaOCl analytical reagent, minimum 5% NaOCl) stock solutions were prepared with deionized water. Stock solutions were kept in lightproof containers to prevent photodecomposition. Measurements of CPO concentration were derived according to procedures outlined in *Standard Methods*.[17] All water samples were taken directly from treatment aquaria at least every 2 to 3 d during each experiment. Agitation of samples was avoided to reduce the potential for error by photodecomposition or flashing-off of CPO.[18] The mixing order of Crecelius et al.[19] (i.e., the addition of 2 mL of pH 4 buffer and 2 mL of 5% KI solution to the sampling cup, followed by the addition of the saline water sample) was used in each titration. Values are reported in milligrams per liter.

3. Ambient water temperature (°C) and salinity (ppt) were measured daily in the incoming seawater from the Stono River in each experiment. A mercury thermometer and a refractometer (American Optics) were used to measure temperature and salinity, respectively. In addition, periodic measurements of dissolved oxygen (Yellow Springs Instrument Company) and pH (Corning pH meter) were made. Dissolved oxygen measurements indicated that oxygen concentrations were always at saturation. The pH ranged from 7.40 to 8.20 during the study.

4. Fecal production or biodeposition rate measurements were made every 2 to 3 d in all treatments by observing the number of oysters producing feces.

5. Respiration was measured on excised gill tissues removed from adult oysters (7–10 cm height) that had been exposed to the various treatments, using methods described in earlier reports.[4,5]

6. *P. marinus* densities in oyster tissues were determined using previously described methods.[20–24] Fifteen adult oysters were removed from each test aquaria on days 0, 15, 30, and 60 of exposure and examined for the *P. marinus* densities. A total of 490 oysters were examined.

The incidence of infection and infection density were determined by counting the number of prezoosporangia in either a 5-mm (50X) or 2-mm (100X) field of view. Three replicate counts of the most heavily infected area of each tissue were made to provide some statistical quantification of infection density.

Infection density code numbers were averaged for each group of oysters to produce the weighted incidence of infection, which is a value reflecting both the proportion of the population infected and the intensity of infection.[8]

Statistical Treatment

Statistical treatment of each experimental measurement in oysters from each treatment included determination of mean, standard deviation, and standard error. T-tests (parametric) and Wilcoxon T-tests (nonparametric) were used to determine if measured differences among various treatments were statistically significant. The 95% confidence level ($p \leq 0.05$) was the minimum statistical significance accepted.

RESULTS AND INTERPRETATION

Salinity Effects

Results of tests comparing the interactive effects of high- and low-salinity exposure on *P. marinus* infection interactions in adult oysters are listed in Tables I through III and depicted in Figures 1A and 1B.

Physical parameters measured in this experiment are listed in Table I. Dilution of seawater with groundwater resulted in low-salinity seawater (E), which had a mean salinity of 9.2 ppt and was well within the desired range of 8 to 10 ppt. Salinity in the ambient high-salinity seawater (A) averaged 22.6 ppt.

Dilution of seawater with groundwater did not significantly lower water temperature, because the mean exposure temperatures in both the high- and low-salinity controls were very similar, 23.9°C in the low-salinity controls as compared with 24.6°C in the high-salinity controls.

Oyster mortalities (Figure 1A) were virtually the same in high and low salinity during the first 33 d of exposure (16 vs 18% mortality, respectively); however, after 60 d of exposure, oyster mortalities were significantly ($p < 0.05$) reduced in the low-salinity treatment by an average of 25% ($\bar{x} = 35\%$ mortality in low salinity compared with 46.7% in high salinity).

Measurements of fecal production or biodeposition rates are listed in Table II. Fecal production was significantly ($p \leq 0.01$) lower in low-salinity controls ($\bar{x} = 64.7\%$) when compared with high-salinity controls ($\bar{x} = 83.4\%$). The lower rate of fecal production in the low-salinity oysters was probably related to the lower phytoplankton density (food availability) in the low-salinity treatment.

Mean gill tissue respiration rates declined throughout the study in both high- ($\bar{x} = 1280.6$ to 1201.5 μL O_2 g^{-1} h^{-1}) and low-salinity ($\bar{x} = 1280.6$ to 1201.3 μL O_2 g^{-1} h^{-1}) controls in response to declining seawater temperatures (28–20°C) (Figure 1B). There were no significant differences in respiration rates measured among oysters in comparing high- and low-salinity controls.

Prezoosporangia densities of *P. marinus* in rectal and labial palp tissues of

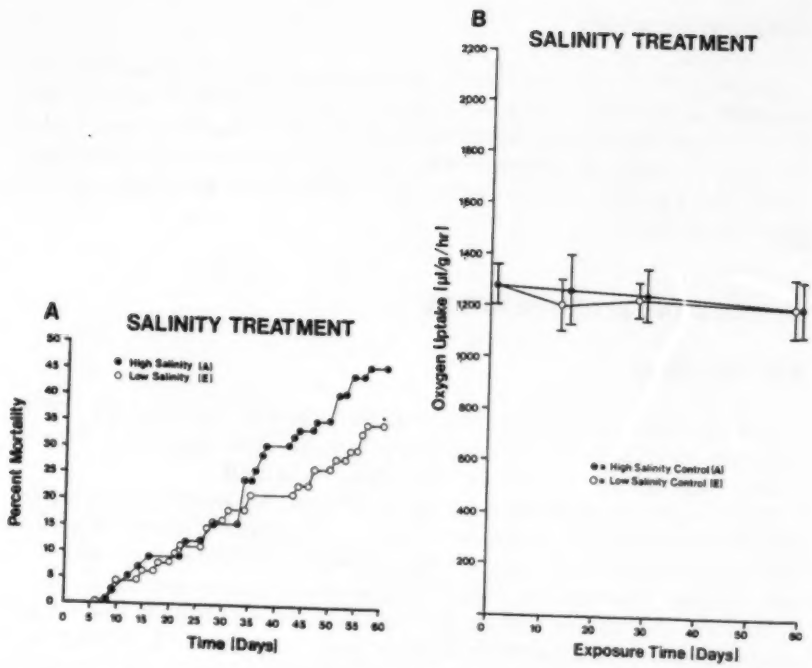

Figure 1. A: Mortality data for oysters exposed to high- (A) and low- (E) salinity treatments. Note that mortality differences between the two treatments were only significant after 30 d of exposure when temperatures fell below 25°C. Asterisk (*) indicates that 60-d mortality results were significantly ($p \leq 0.05$) different; B: Mean gill tissue respiration rates ($\mu L\ O_2\ g^{-1}\ h^{-1}$) measured in oysters exposed to high- (A) and low- (E) salinity treatments. Note the similarity in respiration rates among high- and low-salinity oysters throughout the entire 60 d of exposure. Bars represent sample standard error.

surviving oysters are listed in Table III. The incidence of infection was 100% for both high- and low-salinity-exposed oysters. Prezoosporangia densities in rectal tissues increased throughout the 60 d of exposure in both high- ($\bar{x}$ = 66.23 to 670.18 prezoosporangia per 5-mm field) and low-salinity controls ($\bar{x}$ = 66.23 to 631.14 prezoosporangia per 5-mm field). Prezoosporangia densities in labial palp tissues showed a similar increase in both high- ($\bar{x}$ = 75.8 to 752.9 prezoosporangia per 5-mm field) and low-salinity controls ($\bar{x}$ = 75.8 to 1333.3 prezoosporangia per 5-mm field) throughout the 60 d of exposure. Statistical analysis indicated that prezoosporangia densities were not significantly different in comparisons of rectal and labial palp tissues in oysters exposed to either high or low salinity. Additionally, prezoosporangia densities in rectal tissues were not significantly different in comparisons of high- and

Table II. **Summary of Fecal Production Values Measured in Oysters Exposed to Various Salinity/Chlorination Conditions. Note the significant ($p \leq 0.01$) reduction in fecal production in low-salinity and low-salinity CPO-exposed oysters. Statistical parameters include calculations of mean ($\bar{x}$), standard error (S.E.), sample size (n), and Students T-value. Asterisks (**) indicate sample means were significantly ($p \leq 0.01$) different.**

Treatment	Nominal NaOCl Concentration (mg/L)	Fecal Production (%)				
		Range	$\bar{x}$	S.E.	n	T-Value
A	0.00	60 – 100	83.4	1.54	41	
B	1.00	20 – 100	79.2	1.56	40	
C	0.56	48 – 100	80.3	2.08	40	
D	0.10	64 – 100	82.5	1.65	39	
E	0.00	4 – 100	64.7**	3.61	38	– 4.76[a]
F	1.00	16 – 92	65.5**	2.61	38	– 4.51[b]
G	0.56	12 – 92	72.5**	2.28	39	– 2.53[c]
H	0.10	12 – 92	66.9**	3.19	39	4.34[d]

[a]Calculated from a comparison of treatments A and E.
[b]Calculated from a comparison of treatments B and F.
[c]Calculated from a comparison of treatments C and G.
[d]Calculated from a comparison of treatments D and H.

Table III. Mean Rectal and Labial Palp Hypnospore Densities in Oysters Exposed to Various Chlorination and Salinity Conditions. Hypnospore densities were not significantly different in comparison of high- and low-salinity CPO-exposed oysters or in comparisons with controls. Statistical parameters include calculations of mean ($\bar{x}$), standard error (S.E.), sample size (n), and Student's T-value.

Treatment	Exposure Time (d)	Exposure Temperature (°C)	Rectum Hypnospore Density[a]					Labial Palp Hypnospore Density[a]				
			$\bar{x}$	S.E.	n	T-Value[b]	T-Value[c]	$\bar{x}$	S.E.	n	T-Value[b]	T-Value[c]
A	0	28	77.4	66.23	15			75.8	64.89	15		
A	15	27	61.1	34.74	15			37.5	25.56	15		
B	15	27	2,021.4	1,065.53	15		+1.84	2,007.7	1,068.30	15		+1.84
C	15	27	1,585.2	939.67	15		+1.62	1,495.1	916.13	15		+1.59
D	15	27	81.6	70.77	15		+0.26	62.5	53.88	15		+0.42
E	15	27	3.3	1.41	15	−1.66		2.9	1.24	15	−1.35	
F	15	27	1,058.4	559.58	15	+0.80	+1.89	1,225.2	668.95	15	−0.62	+1.83
G	15	27	2,122.9	1,131.79	15	+0.37	+1.87	1,729.9	998.62	15	+0.17	+1.73
H	15	27	39.5	22.55	15	−0.57	+1.59	32.9	23.04	15	−0.51	+1.30
A	30	24	703.3	692.84	15			753.9	691.70	15		
B	30	24	27.7	7.41	15		−0.98	29.1	15.75	15		−1.05
C	30	24	712.8	697.73	15		+0.01	747.8	697.41	15		−0.01
D	30	24	771.0	713.80	15	+0.70		142.2	85.31	15	−0.88	
E	30	24	14.1	9.00	15		−0.99	10.5	6.07	15		−1.07
F	30	24	1,009.5	726.23	15	+1.35	+0.23	997.7	726.04	15	+1.33	+1.17
G	30	24	795.6	759.27	15	+0.08	+0.02	799.9	76.85	15	+0.05	+0.84
H	30	24	155.3	90.69	15	+1.55	−0.86	130.4	64.92	15	+1.84	−0.11
A	60	20	765.0	670.18	15			752.9	658.44	15		
B	60	20	1,578.1	961.99	15		+0.69	1,508.2	952.28	15		+0.65
C	60	20	546.6	538.36	15		−0.25	696.5	684.71	15		−0.06
D	60	20	800.1	698.15	15		+0.04	809.1	714.65	15		+0.06
E	60	20	702.2	631.14	15	−0.07		1,333.3	902.11	15	−0.52	
F	60	20	2,141.2	1,100.10	15	+0.39	+1.13	1,792.7	945.77	15	+0.21	+0.35
G	60	20	802.9	706.70	15	+0.29	+0.11	659.2	538.94	15	−0.04	−0.64
H	60	20	746.4	724.33	15	−0.05	+0.05	756.1	742.73	15	−0.05	−0.49

[a]Number per 5-mm field.
[b]Comparisons of high-salinity CPO treatments with similar low-salinity CPO treatments (B vs F, C vs G, and D vs H), and comparisons of high- and low-salinity controls (A vs E).
[c]Comparisons of high-salinity controls (A) with high-salinity CPO-exposed oysters (B, C, and D), and comparisons of low-salinity controls (E) with low-salinity exposed oysters (F, G, and H).

low-salinity treatments. Prezoosporangia densities in labial palp tissues were not significantly different in comparisons of high- and low-salinity controls.

Weighted indices of infection-intensity calculations for *P. marinus* in rectal and labial palp tissues are listed in Figure 2. Initially, low-salinity exposure significantly ($p \leq 0.01$) lowered infection intensities in both rectal and labial palp tissues as prezoosporangia densities were substantially reduced during the

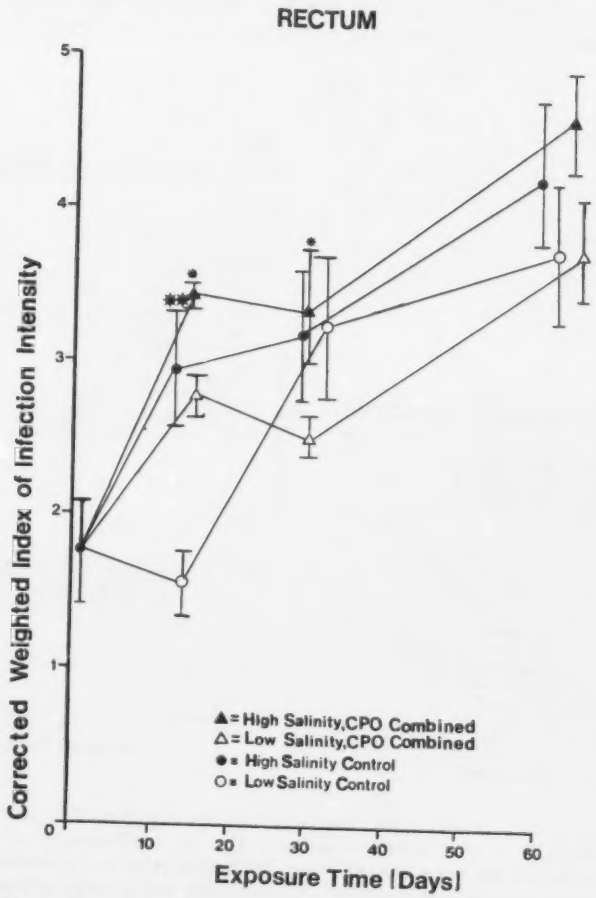

Figure 2. Mean weighted index of infection intensity measured in high- and low-salinity chlorination-exposed oysters. High- and low-salinity control oysters are also included. Values are pooled for surviving oysters and autopsied dead oysters. Asterisks (*) indicate that values were significantly ($p \leq 0.05$) different in comparisons of high- and low-salinity CPO-exposed oysters. Double asterisks (**) indicated values between high- and low-salinity controls were very significantly ($p \leq 0.01$) different.

first 15 d of exposure. However, after 30 d of exposure and continuing through 60 d, infection intensities were practically the same for low- and high-salinity controls in both rectal and labial palp tissues.

Chlorination Treatment

The results of tests comparing the effects of various concentrations of CPO at both low- and high-salinity exposures on *P. marinus* infection rates in adult oysters are listed in Tables I through III and are depicted in Figures 2, 3A, and 3B.

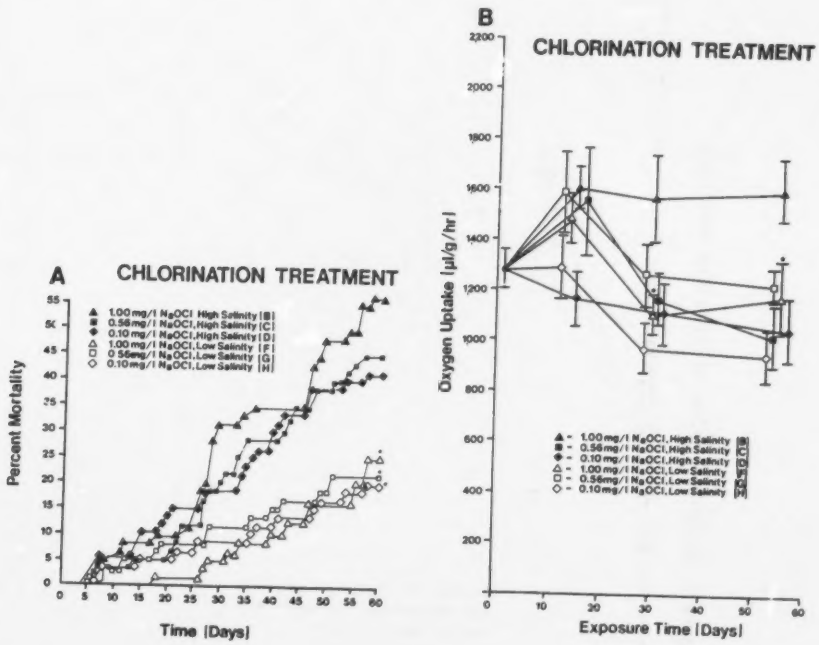

Figure 3. A: Mortality data for oysters exposed to various high-salinity (B, C, D) and low-salinity (F, G, H) chlorination treatments. Note the significantly ($p \leq 0.05$) higher mortality in the high-salinity CPO-exposed oysters. Asterisk (*) indicates that 60-d mortality results were significantly ($p \leq 0.05$) different; B: mean gill tissue respiration rates ($\mu L\ O_2\ g^{-1}\ h^{-1}$) measured in adult oysters exposed to various high-salinity (B, C, D) and low-salinity (F, G, H) chlorination treatments for 60 d. Gill tissue respiration rates generally declined throughout the 60 d of exposure because of declining seawater temperatures. Note the similarity in gill tissue respiration rates between high- and low-salinity CPO-exposed oysters, with the exception of treatment B at exposure days 30 and 60. Asterisk (*) indicates that the 30- and 60-d gill tissue respiration rates in treatment B were significantly ($p \leq 0.01$) higher than in treatment F. Bars represent sample standard error.

Dilution of seawater with groundwater resulted in low-salinity chlorinated seawater with a mean salinity of 9.3 ppt, well within the desired range of 8 to 10 ppt (Table I). Salinity in the ambient, high-salinity chlorinated seawater averaged 22.8 ppt. Mean exposure temperatures averaged 23.6°C in low- and 24.1°C in high-salinity CPO-exposed oyster treatments.

Measured CPO concentrations for each selected nominal NaOCl concentration varied significantly in both high- and low-salinity chlorinated seawater combinations (Table I). Further analysis of these results indicated that measured CPO concentrations for each selected nominal NaOCl concentration were significantly ($p \leq 0.01$–0.05) higher (45–50%) in low-salinity chlorinated seawater exposures (F, G, H) when compared with high-salinity chlorinated seawater exposures (B, C, D).

Oyster mortalities were significantly ($p \leq 0.05$) lower in each low-salinity chlorination treatment when compared with equivalent high-salinity chlorination treatments (Figure 3A). Mean oyster mortalities averaged 22.2%, ranging from 20 to 25% in the various low-salinity chlorination exposures (F, G, H), compared with an average mortality rate of 51.1%, ranging from 41.7 to 56.7% in the various high-salinity chlorination treatments (B, C, D).

Comparisons of mortality rates in the various high-salinity chlorination exposures (B, C, D) with high- and low-salinity controls (A and E, respectively) indicated the mean mortality rate in the nominal 1.00-mg/L high-salinity treatment (B = 56.7%) was significantly ($p \leq 0.05$) higher than the mean mortality rates recorded in high- and low-salinity control oysters (A = 46.7% and E = 35%, respectively). The mean mortality rates in the nominal 0.56-mg/L and 0.10-mg/L high-salinity treatments (C = 45% and D = 41.7%, respectively) were slightly lower but not significantly different from the mortality rate for high-salinity control oysters (A = 46.7%). The mean mortality rates in the nominal 0.56-mg/L and 0.10-mg/L high-salinity treatments (C = 45% and D = 41.7%, respectively) were significantly ($p \leq 0.05$) higher than the mortality rate for low-salinity control oysters (E = 35%).

Comparisons of mortality rates in the various low-salinity chlorination exposures (F, G, H) with high- and low-salinity controls (A and E, respectively) indicated that the mean mortality rates were significantly ($p \leq 0.05$) lower in each of the low-salinity chlorination treatments (F = 25%, G = 21.7%, and H = 20%) when compared to either the high-salinity (A = 46.7%) or low-salinity (E = 35%) controls.

Mortality was lowest (averaging 20%) in the 0.10-mg/L nominal NaOCl concentration, low-salinity treatment (H). This represented a 57% reduction in the mortality rate measured in high-salinity (A) control oysters and a 43% reduction in the mortality rate for low-salinity (E) control oysters.

These findings indicated that the combination of low-salinity (< 10 ppt) and low-chlorine concentrations (0.10–1.00mg/L as nominal NaOCl concentrations) significantly enhanced oyster survival above survival rates measured in both high- and low-salinity regimes, approximating a full salinity range of estuarine conditions.

Fecal production rates were significantly ($p \leq 0.01$) lower in each low-salinity chlorination exposure when compared with equivalent high-salinity chlorination exposures (Table II). Mean fecal production rates averaged 68.3% (ranging from 65.6 to 72.5%) in the various low-salinity chlorination exposures (F, G,H) compared with an average rate of 80.7% (ranging from 79.2 to 82.5%) in the different high-salinity chlorination exposures (B, C, D).

These results indicated that oyster fecal production in the chlorinated high-salinity seawater was significantly higher than in chlorinated low-salinity seawater. Further comparisons with high- and low-salinity control oysters indicated that reduced salinity (or dilution) rather than chlorination had a more profound effect on fecal production, probably related to the lower phytoplankton densities (food availability) in the low-salinity chlorination exposures.

Gill-tissue respiration rates in both high- and low-salinity chlorination exposures initially increased from days 0 to 15, then gradually declined from days 15 to 60 (Figure 3B). Gill tissue respiration rates in high-salinity CPO exposed oysters (B, C, D) generally were not significantly different from values measured in the low-salinity CPO exposures (F, G, H). The only exception to this trend occurred at days 30 and 60 in the 1.00-mg/L nominal NaOCl concentration, high-salinity-treatment values (B = 1568.7 and 1598.3 μL O_2 g^{-1} h^{-1}, respectively), which were significantly ($p \leq 0.01$) higher than corresponding low-salinity chlorination-treatment values (F = 1110.5 and 1179.2 μL O_2 g^{-1} h^{-1}, respectively).

Comparisons of high- (A) and low-salinity (E) controls with the different high-salinity chlorination exposures (B, C, D) indicated that gill-tissue respiration rates generally (in 78% of the samples) were not significantly different between controls and high-salinity CPO exposures. Similar comparisons of different low-salinity CPO-exposed oysters (F, G, H) with high- (A) and low-salinity (E) controls indicated that, generally (in 67% of the samples), gill tissue respiration rates were not significantly different between controls and low-salinity CPO exposures.

The incidence of *P. marinus* infection was 100% for both high- (B, C, D) and low-salinity (F, G, H) CPO-exposed oysters, based on examination of rectal and labial palp tissues (Table III). Prezoosporangia densities were highly variable in all CPO and salinity exposures; however, these densities generally increased in both rectum and labial palp tissues throughout the 60 d of exposure in both high- and low-salinity CPO-exposed oysters. Statistical analysis for high- (B, C, D) and low-salinity (F, G, H) CPO-exposed oysters indicated that there were no significant differences in comparisons with high- and low-salinity chlorination treatments. Comparisons within each salinity and chlorination treatment indicated that rectal prezoosporangia densities were generally greater than labial palp tissue densities.

Additional comparisons of high- (B, C, D) and low-salinity (F, G, H) CPO-exposed oysters with high- (A) and low-salinity (E) controls indicated no significant differences between controls and the various chlorination treatments in prezoosporangia densities of rectal and labial palp tissues.

Weighted index of infection-intensity calculations for *P. marinus* in rectal tissues are shown in Figure 2. Infection intensity generally increased throughout the 60 d of exposure in both rectal and labial palp tissues of high- (B, C, D) and low-salinity (F, G, H) CPO-exposed oysters. This same trend was observed in high- and low-salinity control oysters.

Statistical analysis of high- (B, C, D) and low-salinity (F, G, H) CPO-exposed oysters indicated there were significant ($p \leq 0.05$) differences in comparison of the weighted index of infection intensities among rectal tissues at days 15 and 30 of exposure. Comparisons of high- (B, C, D) and low-salinity (F, G, H) CPO-exposed oysters with high- (A) and low-salinity (E) controls indicated there were generally no significant differences in rectal infection intensities among controls and the various salinity and chlorination treatments. In similar comparisons of labial palp tissue, there were generally no significant differences among controls and the various salinity- and chlorination-treated oysters.

DISCUSSION

Results from this study have generally indicated the significant interactive effects of salinity and CPO exposure in affecting oyster mortalities resulting from *P. marinus* infection.

Comparisons of control oysters in high- and low-salinity exposures generally indicated that low-salinity conditions resulted in exposure of oysters to water which was reduced in both salt content (major ions) and phytoplankton density (as food availability was reduced by 66%). Biodeposition rates (or feeding rates) were significantly reduced in low-salinity-exposed oysters, probably related to less food availability. Galtsoff[25] reported similar reduced feeding rates in oysters exposed to low salinity. Exposure to low-salinity conditions and accompanying reduced fecal production rates did not significantly affect oyster physiology, because measurements of condition index, gonadal index, and gill tissue respiration rates were not significantly different (and were generally identical) throughout the 60 d of exposure.[26]

Analysis of mortality data from high- and low-salinity control conditions indicated that low-salinity exposure significantly increased oyster survivability of *P. marinus* infections by 25%. This significant increase in survival occurred in low-salinity control oysters even though there were generally no statistically significant differences in the incidence of infection, prezoosporangia density, and weighted index of infection intensity in comparisons of high- and low-salinity control oysters. Analysis of the weighted index of infection-intensity data suggested that low-salinity exposure initially decreased infection intensity (at day 15); however, by days 30 and 60, infection intensities were equal for both high and low salinities. Earlier studies[9,12] have indicated similar delays in prezoosporangia development from low-salinity exposure.

Although infection-intensity differences in comparisons of high- and low-salinity control oysters were only slight, actual differences may have been

obscured by the higher mortality rate in high-salinity control oysters, because the most heavily infected oysters were presumably dying off first (this can be presumed since measurements were not made of gapers but rather of living specimens). The death of the most diseased individuals would result in the selection of more-resistant or less-infected oysters, which would tend to bias infection intensities downward to much lower levels than if all heavily diseased or dead individuals were included.

The mode of action for low-salinity exposure in significantly reducing oyster mortalities has been reviewed by several authors,[9,11] who generally concluded that low-salinity exposure must enhance survival because of the possible reductions in the number of waterborne prezoosporangia that would tend to limit and reduce both the incidence and intensity of infection. However, the fact that all oysters in this study were infected with *P. marinus* at day 0, since the infection spreads only from the initial site of infection, suggests that differences in survival were not related to differences in prezoosporangia density in seawater but rather to physiological differences in oysters exposed to different salinity.

Results from CPO exposure treatment indicated that the combination of low salinity and CPO exposure significantly ($p \leq 0.05$) reduced oyster mortalities by 47 to 57% below high-salinity controls at all concentrations tested (0.10–1.00 mg/L nominal NaOCl concentration; measured CPO concentrations of 0.02–0.11 mg/L). The combination of high salinity and CPO exposure increased mortality by 21% above high-salinity control levels at high chlorination doses (1.00 mg/L nominal NaOCl concentration; measured CPO concentration of 0.06 mg/L), whereas in lower concentrations (0.56- and 0.10-mg/L nominal NaOCl concentrations; measured CPO concentrations of 0.03 and 0.01 mg/L, respectively) mortality was equal to or slightly lower than high-salinity control mortality rates. Earlier studies[4,5] reported similar increased mortality 14% above control values in oysters exposed to a 1.00-mg/L nominal NaOCl concentration in high-salinity waters during the same time period of the year.

Additional comparisons indicated that the combination of low salinity and CPO exposure significantly reduced oyster mortality below low- and high-salinity control values.

Fecal production measurements indicated increased feeding rates in high-salinity CPO-exposed oysters (79.2–82.5%) when compared to low-salinity CPO-exposed oysters (65.5–72.5%). Fecal production rates in high- and low-salinity CPO-exposed oysters were not significantly different from rates for each respective high- and low-salinity control. Decreased fecal production was considered attributable to low-salinity effects rather than CPO exposure.

These results differ significantly from earlier[4–6] studies that reported CPO exposure caused significant reductions in fecal production rates at low chlorine concentrations (nominal 1.00 mg/L NaOCl). These earlier studies were continuous-exposure flow-through experiments as compared with the intermittent (8–12 h/d CPO exposure) conditions used in this study.

Comparisons of gill tissue respiration rates in high- and low-salinity CPO-exposed oysters generally indicated that respiration rates were slightly lower in low-salinity CPO-exposed oysters. Gill respiration rates were only significantly ($p \leq 0.01$) different in comparison with 1.00-mg/L nominal NaOCl concentration, high-salinity-treatment oysters and high-salinity control oysters, which indicated increased oyster respiration rates in the 1.00-mg/L nominal NaOCl concentration, high-salinity exposure treatment. Earlier studies[4-6] have reported similar increased gill tissue respiration rates in oysters chronically (60 d) exposed to 1.00-mg/L nominal NaOCl concentrations under similar exposure conditions, which resulted from increased anaerobic metabolism by oysters to survive CPO exposure.

Results from this study suggested that CPO exposure at similar chlorine concentrations (1.00-mg/L nominal NaOCl) increased gill tissue respiration rates, but only under high-salinity conditions. Higher gill tissue respiration rates resulted from increased reliance on gonadal glycogen reserves to survive CPO exposure under high-salinity conditions. Absence of these effects in lower CPO concentrations under high-salinity exposure and in low-salinity CPO exposures implied that CPO exposure must interact with factors representative of normal high-salinity exposure conditions per se, such as increased *P. marinus* infection intensity, to cause increased stress in these oysters. Such interaction would result in a more rapid depletion of stored gonadal glycogen reserves because of the increased metabolic utilization of glycogen by the oyster to survive CPO exposure and by the parasite as the infection intensifies and spreads. There was a rapid decline of gonadal indices in high-salinity CPO-exposed oysters in this study, which may be indicative of this effect.[26] The rapid depletion of gonadal glycogen stores resulting from this combined host-parasite effect would probably result in earlier utilization of protein stores (amino acids) as glycogen levels declined. This would signify utilization of different metabolic pathways (nitrogen), both by the host (oysters) and parasite.

Consideration must be given to physiological and biochemical differences between high- and low-salinity control oysters. Oysters are euryhaline organisms surviving wide ranges of salinity as low as 5 to 7 ppt.[25,27] Additionally, oysters are considered osmoconformers that prefer salinities of 11 to 30 ppt.[25,28] Osmoregulation in low-salinity seawater appears to result from volume regulation, the result of intracellular solute loss.[29] The source of these intracellular solutes has been reported to be the intracellular, free-amino-acid pool. Decreased levels of taurine, alanine, glycine, and proline have been reported in intertidal bivalve species, whereas reduced levels of taurine, alanine, and glycine have been reported in subtidal bivalve species.[29] Under low-salinity exposure, free-amino-acid levels may decline by as much as 84%, particularly ninhydrin positive (i.e., glycine and alanine) substances.[30,31] Low-salinity exposure would significantly reduce the amount of intracellular, free amino acids in oysters during this study.

This reduction of free-amino-acid levels in low-salinity control oysters may,

perhaps, explain the higher survival observed under low-salinity exposure. Low-salinity oysters may have increased survival because of the reduced availability of protein or amino acids as food substrate for *P. marinus*, particularly if the infection intensifies to heavy intensities.

Additional consideration must be given to results of studies by Soniat and Koenig.[32] They found that free-amino-acid levels in oysters declined with increasing levels of *P. marinus* infection. This suggests increased use of free amino acids by both the host (oyster) and parasite during periods of infection. Soniat and Koenig[32] also reported increased levels of taurine and aspartic acid with increased infection intensity, although total free-amino-acid levels declined with increasing infection intensity. Lange[33] similarly reported increased levels of free amino acids with increased salinity in *Mytilus edulis*, with taurine having the most dramatic increase with increased salinity. Giles[34] reported a 55% decrease in taurine and an 82% decline in aspartic acid levels in *M. edulis* exposed to low-salinity seawater. These studies suggest that low-salinity exposure may reduce free-amino-acid levels as the oyster osmoregulates in response to salinity stress. This response may reduce the use of the free amino acids by the parasite, thus limiting the extent of infection and perhaps enhancing oyster survival.

Exposure of oysters to low salinity and low-salinity CPO conditions probably resulted in oysters that had lowered levels of both glycogen and free amino acids. This would initially tend to retard secondary spread and intensification of the disease; however, the fact that infection intensities were generally equal among all treatments after 60 d of exposure, and that mortality was generally higher in high-salinity control oysters and high-salinity CPO-exposed oysters, may suggest that the lowered levels of free amino acids in low-salinity control oysters and low-salinity CPO-exposed oysters offered some degree of protection to the oyster by preventing the disease from becoming toxic. Further research in this area is needed to better understand the exact mechanism of host-parasite interactions to chlorine as well as to other environmental pollutants.

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